JBP 246
PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Charles E. Clum et al.

Serial No.: 700,165 Group No.: 125

Filed : February 11, 1985 Examiner : J. Lipovsky

For : SKIN CARE COMPOSITIONS

DECLARATION UNDER RULE 132

Honorable Commissioner of Patents and Trademarks
Washington, D. C. 20231

Dear Sir:

I, BRUCE SEMPLE, declare and say:

THAT I received a Medical Degree from the University of Sydney, Australia in 1959 and served residencies in general and orthopedic surgery;

THAT I had a private family and surgical practice and served clinical assistantships in orthopedics, surgery and endocrinology in various Australian hospitals;

THAT from 1967 to 1974, I served as Medical Director and then Vice President Medical Affairs for Miles Laboratories, Inc.;

THAT I have been employed since 1974 by Johnson & Johnson Baby Products Company serving as Director of Medical and Regulatory Affairs from 1974 to 1979, as Vice President - Research and Development from 1979 to 1984 and presently as Vice President - Scientific Affairs and member of the Board of Directors;

THAT I am familiar with the invention disclosed and claimed in United States Patent Application Serial No. 700,165 and further that I am familiar with the Office Actions relating to said application and that I previously submitted a Declaration Under Rule 132 dated March 7, 1986 in connection with said application;

THAT under my direction as Vice President - Research and Development of the Johnson & Johnson Baby Products Company in 1982, I authorized studies of the ability of various diaper rash formulations to inhibit the growth of Candida albicans on human skin to be conducted by Dr. James J. Leyden at Ivy Research Laboratories, Inc.;

THAT the test procedure was as follows: Ten subjects (four females and six males) with an age range of 19 to 27 years were randomized into a controlled double-blind study to compare the ability of four treatments to prophylactically inhibit the growth of Candida albicans on the arms of the subjects. Six test sites on the forearms of each subject were randomized according to the following treatment plan:

a) Base (no actives)
b) 15% zinc oxide in base
c) 0.25 miconazole nitrate in base
d) 0.25 miconazole nitrate and 15% zinc oxide (1:60 ratio) in base
e) no treatment

Twenty microliters of a saline suspension of Candida albicans containing 1 million cells per milliliter are applied to three one square centimeter test sites on the volar forearm surface of each volunteer subject. The areas are covered with an impermeable plastic film and secured with tape. The test sites

are uncovered six hours after inoculation and treated with one of the test products or left untreated as a control. The sites are then redressed with plastic film for an additional 24 hours. The test products are then removed from all sites and cultures are obtained by the standard detergent scrub method of Williamson and Kligman. This method is set forth in the Journal of Investigative Dermatology, Vol. 45, No. 6, pps. 498-503 (1965); a copy of which was included with my prior Declaration Under Rule 132;

THAT two different types of measurements and data were obtained from these tests; microbiological measurements and clinical measurements;

THAT the microbiological data was obtained as follows: cultures from all test sites collected as above were prepared on Trypticase Soy Agar and Littman Media plates and incubated for 48 hours. Colonies were then counted by standard microbiological methods;

THAT for each of the counts, miconazole alone gave lower counts than zinc oxide alone, but these difference were not statistically significant. For Candida albicans on Trypticase media, miconazole alone gave lower counts than the base, while the remaining products do not differ. For Candida albicans on Littman media, counts with miconazole alone and zinc oxide alone were lower than the base, but not different from each other or the untreated site. Results for the miconazole nitrate and zinc oxide product were significantly lower than all the other products. To determine if the relation between zinc oxide and miconazole was additive or synergistic, a second analysis of variance was performed without the untreated site. The model included zinc oxide, miconazole and the interaction of these ingredients. The results show a small but statistically significant synergistic effect;

THAT these microbiological results can also be represented in tabular form as follows:

Synergistic Activity of Zinc Oxide on Miconazole Nitrate
Inhibition of the Growth of Candida Albicans at
Miconazole Nitrate Concentration of 0.25 w/v

Zinc Oxide Concentration 15% w/v	Inhibition for Miconazole Nitrate (%)	Inhibition for Zinc Oxide (%)	Sum of Components %	Inhibition for Combination %
Trypticase Media	13.8	5.5	19.3	25.0
Littman Media	15.4	7.6	23.0	25.0

THAT as stated above, these results exhibit a statistically significant synergistic effect. This synergistic effect cannot be attributed to experiemental error because of the precision of the experiment itself and particularly the microbiological counts. The probability of an experimental error producing this effect is $p<0.05$. Therefore, the inhibition for the combination, i.e., miconazole nitrate and zinc oxide, in both the Trypticase media and the Littman media demonstrates synergism;

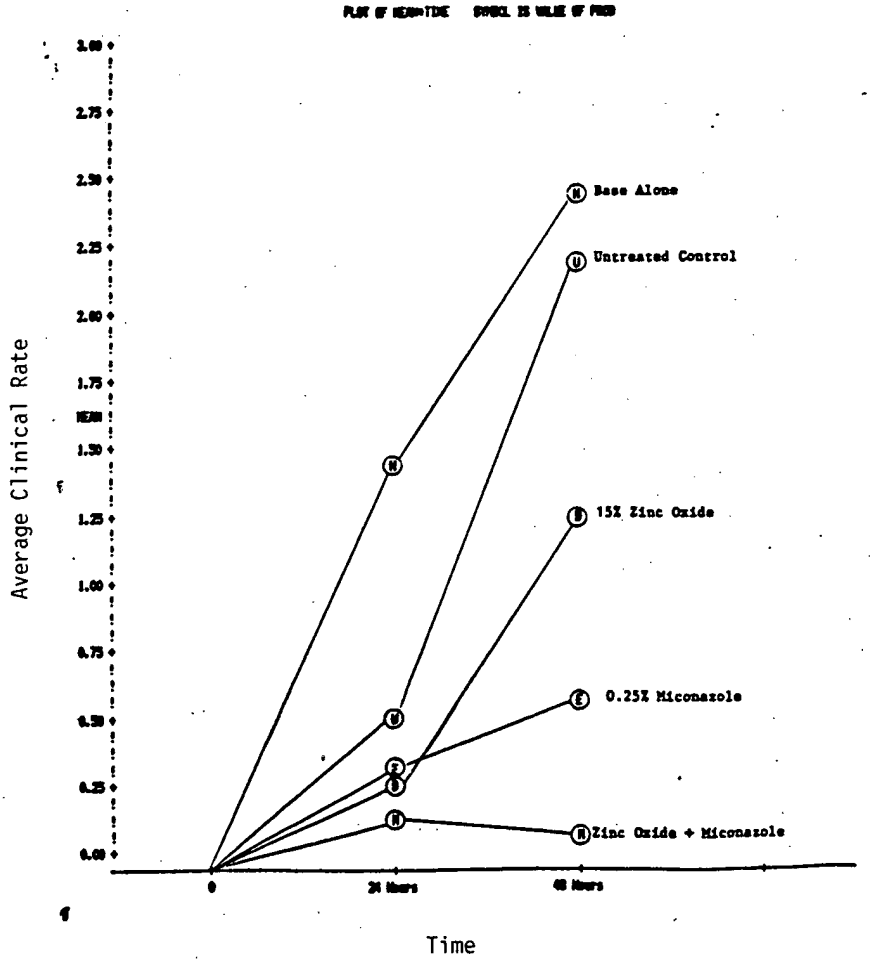
THAT the clinical data was obtained as follows: measurements were obtained by experienced investigators for each site immediately prior to treatment, at 24 hours and at 48 hours after treatment. Grading is done on a scale of 0=no reaction, 1=minute pinpoint papules and/or faint erythema, 2=at least 5 discrete papules or pustules and definite erythema, 3=greater than 10 papules or pustules and erythema, 4=confluent papules or pustules and intense erythema;

THAT the following clinical results were obtained: at 24 hours, reactions at the sites treated with base only were more severe than the other treatments, while the other products did not differ significantly. By 48 hours, the clinical severity

had increased with all treatments except the zinc oxide-miconazole product. The zinc oxide-miconazole product severity was directionally less than miconazole alone, and significantly less than all other treatments both by chisquare tests and two-way analysis of variance;

THAT one could conclude from these tests that the clinical scores 48 hours after inoculation demonstrated that the combination of miconazole nitrate and zinc oxide was superior to miconazole alone and superior to the other treatments. Furthermore, the combination produced significantly lower counts of Candida albicans than all other treatments;

THAT these clinical results can be represented in graphical format as follows:



THAT these clinical results are statistically and clinically significant and particularly so when based on the experience of an investigator of the renown of Dr. Leyden;

THAT, in summary these results demonstrate, both clinically and microbiologically, that the combination of 0.25% miconazole nitrate in a base containing 15% zinc oxide is superior to miconazole alone and the base and indicate their synergistic effect;

THAT in my opinion, the above described test procedures and results therefrom clearly establish the efficacy and synergistic nature of miconazole nitrate - zinc oxide combinations in a 1:60 ratio against Candida albicans.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Subscribed this day of August, 1986.

Bruce Semple

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